CYTOLOGY OF SACCHAROMYCES CERVICIÆ WITH ESPECIAL REFERENCE TO NUCLEAR DIVISION.

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For some years following the discovery of cell division it was thought that direct division was responsible for the duplication of the nucleus, and even after the discovery of indirect division the former was still considered to be the usual method and mitosis was held to be a peculiar and infrequent phenomenon. Some held that the two processes were related and that mitosis was derived from the simpler and more primitive one, amitosis. However, as the investigation of this problem was continued evidence was gradually accumulated to show that mitosis is the usual if not universal method of cell division in higher animals and plants, and that amitosis is not a reproductive phenomenon at all, but has for its function the increase of nuclear surface in relation to volume and is rarely, if ever, followed by cytoplasmic division. This conception was well expressed by Conklin (1917) in the following passage, "Mitosis and amitosis are fundamentally unlike. Mitosis is the one and only method of bringing about equal division and distribution of the chromatic material of the nucleus. Amitosis is not a genuine divisional phenomenon at all, but merely a means of increasing the nuclear surface and of distributing nuclear material throughout the cell, comparable to nucleur lobulation, fragmentation or distribution. These two processes are not equivalent or even comparable, nor may one of them be converted into the other." Although this view is not universally held it is very generally accepted by cytologists at the present time.

The study of cell reproduction is much more difficult in Protozoa than in higher forms, as many of the types of intranuclear division have a superficial resemblance to amitosis. Minchin (1912) actually accredits the description of direct division in a number of protozoa, although he admits that some

of the formerly supposed cases of amitosis have been shown to be merely simulations of it. Some of the authorities in this field, however, are willing to elevate the Protozoa to essentially the same position as Metozoa in this respect. Kofoid (1923) says: "In the first place amitosis as described in the Protozoa is either a pathological or degenerative process, as it is in the Metozoa, or it is based on a partial account of the normal process of mitosis in which the nuclear membrane remains intact throughout the whole process, as it does in the flagellates and rhizopods, and in its anaphases presents a superficial resemblance to pathological amitosis. The persistence of the nuclear membrane in no way interferes with the occurrence of chromosomes constant in number and kind. In other words the doctrine of chromosome continuity, in so far as amitosis is concerned, is no more affected in the Protozoa than in the Metazoa." The nuclear division (promitosis) of many of the lower protozoa is unquestionably very different from the mitotic division exhibited by higher plants and animals, but it seems likely that in all cases it is a simplified form of mitosis and is entirely unrelated to amitosis, even though it does superficially resemble it.

It seems as though the difficulty of studying nuclear division in any form is responsible for the persistence of investigators in describing it as amitotic. The tapeworm *Monezia* offers an example of this (Child, 1911). Yeast belongs in the same category, and we should realize the extreme difficulty of investigating this problem in yeasts by considering the minute size of the cells and the fact that for some time there was a heated controversy as to whether or not they even possess a nucleus. Some of those who took the affirmative, as has since been proved, were describing structures which belong to the cytoplasm. Wager (1898) and Wager and Peniston (1910) described as the nucleus, the actual nucleus, the vacuole, and a part of the metachromatic material surrounding the nucleus and vacuole. The division of this compound structure was described as amitotic in the case of budding and by an "intermediate step in karyokinesis" in the case of spore formation. This account of the indirect division of a cytoplasmic vacuole is comparable to the early figures of mitosis of the parabasal body (kineto-

nucleus) in trypanosomes and shows that without a favorable modification of technique the problem is almost invincible.

Some of the early contributors to our knowledge of the cytology of yeasts unquestionably saw and illustrated, with a fair degree of accuracy, not only the resting nucleus but also stages in its division. Janssens (1902) and Janssens and Leblanc (1898) considered the division of the nucleus to be an intermediate form of mitosis. Swellengrebel (1905) and Fuhrmann (1906) as a true mitotic process. Although these last two articles are in the main correct they have not been generally approved, and the ideas of Guilliermond (1904, '12, '17 and '19), which gain weight by the mere bulk of his work on yeast, seem to meet with more favor. This author, who is responsible for a large part of our knowledge of the well developed sexuality of yeasts and for an excellent account of the typical metazoan mitosis found in spore formation in *Schizosaccharomyces octosporus*, maintains that in bud formation the nucleus divides by a process identical with amitosis in the tissue cells of higher organisms, where, as Conklin asserts, it is not a reproductive phenomenon at all. To accept this would be to admit that mitosis and amitosis are fundamentally alike and interchangeable. This would undermine a large part of our knowledge of cytology and genetics. The problem most assuredly warrants critical study.

Methods.

Pure cultures of *Saccharomyces cerviciæ* were used for this work. They were cultivated on both liquid and solid media. French proof broth was used for the liquid medium, French proof agar for the solid.

The organisms were transferred to slides which had been previously smeared with albumen fixative and the moist films were fixed either in corrosive-acetic-alcohol (95 per cent. alcohol saturated with mercuric chloride 95 parts, glacial acetic acid 5 parts) or Bouin's solution (saturated picric acid solution 75 parts, formalin 20 parts, glacial acetic acid 5 parts). Iron-alum-hæmatoxylin counterstained with light green or not counterstained at all was found to be the best means of staining. Delafield's hæmatoxylin and carbol fuchsin were tried without

success, and eosin and orange G were found to be equally useless.

Fixation in corrosive-acetic-alcohol slightly shrinks the cells and gives the chromatic material in the cytoplasm such great affinity for basic stains that in most cases the nucleus is obscured. After fixation in Bouin's it does not stain so heavily and the nucleus can be seen in all cases, providing it is not too heavily stained. The successful technique finally developed was fixation in Bouin's and staining before the picric acid was completely washed out. This decreased the affinity of both nuclear and cytoplasmic chromatic material for stain, especially the latter. In this way the nucleus could be stained without showing the chromatic bodies in the cytoplasm at all. This procedure made possible a careful study of the nuclear chromatin both at rest and during division. Light green was found very valuable for the study of the metachromatic granules. Even when they are very abundant this stain will demonstrate their granular nature. In preparations in which no counterstain or the other acid stains are used they frequently appear as a single, large mass.

It was found that fixation and staining in carbol fuchsin followed by light green furnished an excellent method for demonstrating the alveolar nature of the cytoplasm. Although the nucleus could be seen in these cells its minute structure was not visible and the method is of no value for the present work. Light green is taken up by the gelatinous secretion sometimes given off by the yeast cells and consequently furnished a good means of demonstrating the presence of this substance.

Morphology.

Saccharomyces cerviciæ is a round to slightly oval yeast whose size ordinarily ranges from 5 to 10 micra. In old cultures the cells are occasionally elongated, sometimes crescentic (Fig. 7). The cell is enclosed in a thin though distinctly evident wall. This wall occasionally becomes greatly thickened and the cells pass into a very resistant stage (durable cells, chlamydospore) such cells can be kept dry for a year without destroying their viability (Figs. 10 to 13).

One of the most prominent structures inside the cell is the

vacuole, small or entirely absent in very young cultures, but attaining a relatively great size after several days. In some cases it almost entirely fills the cell (Fig. 2). This vacuole is generally round, with a very regular outline. The cytoplasm is distinctly alveolar and contains numerous metachromatic granules. Occasionally cells are found with none of these granules, especially in very young cultures (Fig. 5) and sometimes there are only a few present, in which case they are located near the nucleus (Fig. 4). In old cells with a large vacuole there is frequently such a great mass of metachromatic material surrounding the nucleus that the latter body is obscured (Figs. 2 and 3), and the granular nature of the metachromatin is not discernible. Lines of fine granules can be seen leading from this mass to large granules in other parts of the cell, thus converting the metachromatic material into a connected unified system. These granular strands are especially evident where they go around the vacuole, and constitute the nuclear reticulum of Wager. Guilliermond figures basophilic granules within the vacuole. It is these granules above and below the vacuole which he sees and there are really no granules within the large vacuole. When material is fixed in Bouin's solution and stained in iron-alum-hæmatoxylin without washing out the picric acid the metachromatic granules do not stain. When this procedure is properly carried out, the well stained nucleus embedded in the alveolar cytoplasm is quite evident and easily studied. (Figs. 6 to 8.)

The nucleus of *Saccharomyces* is quite similar to that of higher plants, particularly *Phaseolus* (Kater, 1926). The nuclear membrane is equally as evident as in higher plants. Centrally located is a large basophilic nulceolus or karyosome from which radiate slender slightly basophilic linin strands. These strands run from the nucleolus to the nuclear membrane in identically the same manner as in *Phaseolus*. There are generally about six such strands visible. Just inside the nuclear membrane are located a number of chromatin granules. The larger ones are found at the points where the linin strands come in contact with the nuclear membrane. Except for the minute size of the nucleus (1 to 3 micra in diameter) it is hardly distinguishable

from that of *Phaseolus*. A fruitful study of the finer points of the structure of the resting nucleus as well as following it through division would have been impossible without some means of staining it without affecting the metachromatin. It is this feature of our technique which makes the observations convincing. A centrosome could not be identified in the interkinetic cell nor could any variation in the cytoplasm surrounding the nucleus, comparable to sphere substance, be seen.

The yeast cell contains both fat and glycogen, the former usually in small globules in the vicinity of the nucleus.

It has been previously observed that yeast cells will give off a gelatinous secretion when permitted to dry gradually in a closed vessel. This is thought to play a part in the agglutination of yeast and the consequent clearing of the medium. In the present work it was found that dilution of the medium of an old culture with distilled water will produce this secretion (Fig. 9). The secretion has great affinity for light green.

Budding.

In young cultures the usual method of reproduction is by budding. The superficial features of this process are matters of common knowledge, and consequently, this description will be limited to the internal phenomena. The bulge in the cell wall and the entrance of cytoplasmic elements into the bud occurs some time before any change in the resting nucleus is observable (Fig. 14). The finely vacuolated cytoplasm is the first material to enter the bud. This is followed by the metachromatic granules. In those cells that contain a large vacuole the bud is generally formed near the nucleus and consequently near the greater part of the metachromatic material. The mass of this material near the bud separates into individually visible granules, part of which migrate through the isthmus into the bud, the rest moving to the opposite side of the parent cell (Figs. 15 and 21). Whether or not they divide at this time cannot be stated. Even after these granules are distributed to the two cells the nucleus is still in the resting condition. The large clear vacuole does not divide, but after the bud has attained almost the size of the parent cell a small vacuole appears within it and gradually enlarges.

After the bud has attained almost normal size and the distribution of metachromatic granules is completed the nucleus gives evidence of impending division. The chromatin accumulates on the linin strands, making them very evident and extremely basophilic. At the same time the disperse chromatin granules enlarge and the whole nucleus becomes a perfect miniature of an early prophase in *Phaseolus vulgaris* (Kater, 1926, Figs. 15 and 16). Meanwhile the nucleus remains stationary (Fig. 16). The steps in the transformation of such a nucleus into chromosomes cannot be followed with any degree of certainty, but many stages such as the one represented in Fig. 17 were seen. A knot of chromosomes probably occupying the old location of the nucleolus is here observed at the periphery of which individual chromosomes are becoming disentangled from the rest.

So far neither a dividing centrosome or spindle can be identified. However, as the chromosomes become arranged on the metaphase plate a spindle becomes visible (Fig. 18) and in some cases the ends are lodged in a granule perfectly comparable to the centriole in metazoa. No astral radiations are visible (Fig. 19). Such a centrosome has previously been reported in yeast (Swellengrebel, 1905; Guilliermond, 1917). In Fig. 18, the chromosomes are quite clearly separating in the same way as they do in higher plants and animals. Except for the difference in size the early anaphase represented in Fig. 19 could easily be mistaken for a metazoan mitotic figure. No careful attempt was made to count the chromosomes of *Saccharomyces* as their size makes it impractical. However, it seems certain that there are more than the four reported by Swellengrebel (1905) and Fuhrmann (1906). Probably at least twice that number.

The migration of the chromosomes through the isthmus into the bud is not at all clear. In some figures the orderly arrangement of the anaphase plates seems to be maintained (Fig. 22) while in others the chromosomes appear to enter the bud one at a time. In such cells the spindle disappears and only the irregularly arranged chromosomes can be seen (Fig. 21). In overstained slides specimens similar to the one illustrated in Fig. 22 very greatly resemble amitosis, and are probably responsible for the confusion on this point.

After being distributed to the two daughter cells the chromosomes become collected at one point and apparently fuse forming a uniformly basophilic mass in which the individual chromosomes cannot always be identified. The imbibition of achromatic material results in the alveolization of the outer part of this mass. The limiting membrane (nuclear membrane) is frequently bulged between the linin strands (Fig. 25). A continuation of alveolization results in a spherical nucleus with a nucleolus of moderate size, the resting condition.

The division of the cytoplasm in the isthmus occurs shortly after the separation of the chromosomes and the disappearance of the spindle (Fig. 24). Although the cytoplasm has separated, the cell wall frequently does not divide for a considerable length of time and holds the two cells together. In this way a number of cells are sometimes connected, somewhat resembling a mycelium (Fig. 8).

No attempt has been made to study the formation of ascospores, but according to previous accounts the nucleus divides by mitosis.

Discussion.

The above account leaves two points without adequate solution, namely the transformation of the nucleus into chromosomes and the migration of chromosomes through the isthmus. Fortunately these are matters of detail and the remainder of the account shows conclusively that the nucleus of yeast, in the formation of buds, does not divide by constriction, but that chromosomes are formed, divide (Fig. 18), separate, and give rise to daughter nuclei in the same way as in higher organisms, and yeast can be placed in the same category with higher animals and plants, and protozoa as enunciated by Conklin and Kofoid respectively.

To accept Guilliermond's descriptions of the indirect division of the nucleus in the formation of ascospores and direct division in budding would be to admit the reproductive nature of amitosis and, secondly, that a nucleus produced by this method may later divide, in the ascus, by perfectly normal mitosis. This would make untenable many of the generalized conceptions of

the cytologist and the geneticist. It seems remarkable that the investigation of nuclear division in yeast has not previously attracted the attention of cytologists, as well as mycologists.

In the earlier work on *Phaseolus* it was found that the linin strands radiating from the nucleolus are actually the linin sheaths of chromosomes which persist through the resting condition and give the chromosomal vesicles morphological individuality during interkinesis. It seems probable that the linin strands of *Saccharomyces* are of the same nature. The collection of chromatin along them in the early prophase (Fig. 16) and the bulge between them in the telophase (Fig. 25) would indicate this. However, since the actual transformation of the prophase nucleus into chromosomes and the alveolization of early telophase chromosomes could not be followed with any degree of certainty a definite statement of chromosomal continuity in yeast cannot be made on a morphological basis as in *Phaseolus*, but the probable homology is certainly worthy of mention.

The existence of radiating linin strands alone would not justify this interpretation, since they are present in many protozoan nuclei where the nuclear membrane remains intact throughout mitosis. In such organisms the membrane is not a product of the linin sheaths of chromosomes, as in higher animals and plants, and the linin strands could not easily be interpreted in the same manner. Such a nucleus is found in Polytomella citri (Kater, 1925). In *Saccharomyces* the nuclear membrane disappears and consequently it is quite possible that the linin strands are homologous with those of *Phaseolus*.

Summary.

The nucleus of *Saccharomyces cerviciæ* divides by mitosis in the process of budding.

The chromosomes apparently form, divide, separate, and give rise to daughter nuclei in much the same way as in *Phaseolus*. The linin strands connecting the nucleolus and nuclear membrane probably represent sheaths of chromosomal vesicles.

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EXPLANATION OF PLATES.

Figures 1 to 5 and 15, 20, and 21 made from material fixed in corrosive-acetic-alcohol and stained with iron-alum-hæmatoxylin, Figs. 4, 5, and 15 counterstained with light green. The remaining figures from material fixed in Bouin's solution and stained as above. All drawings made with Abbe model camera lucida. Magnification 3,200 ×.

PLATE I.

FIG. 1. Small yeast cell showing fine granular lines of chromatic material in the cytoplasm, nucleus partly obscured.

FIG. 2. Nucleus entirely obscured. Granular lines above vacuole clear.

FIG. 3. End view of a cell similar to Fig. 2.

FIG. 4. Cell containing only a few metachromatic granules. Nucleus visible. Medium-sized vacuole.

FIG. 5. No metachromatic granules. Nucleus very large and structure clear.

FIG. 6. Nucleus above vacuole which is not so clear as in side view.

FIG. 7. Cell exhibiting crescentic form.

FIG. 8. Four connected cells showing manner of connection by unbroken wall.

FIG. 9. Cross hatching indicates gelatinous secretion which holds the cells together.

FIG. 10. Resistant cell. Note heavy wall and abundance of metachromatic granules.

FIG. 11. The same. Metachromatic granules disappeared.

FIG. 12. Resistant cell showing departure from spherical form and shrinking of protoplast.

FIG. 13. Resistant cell that has been kept dry for one year.

Drawings by G. T. Kline.

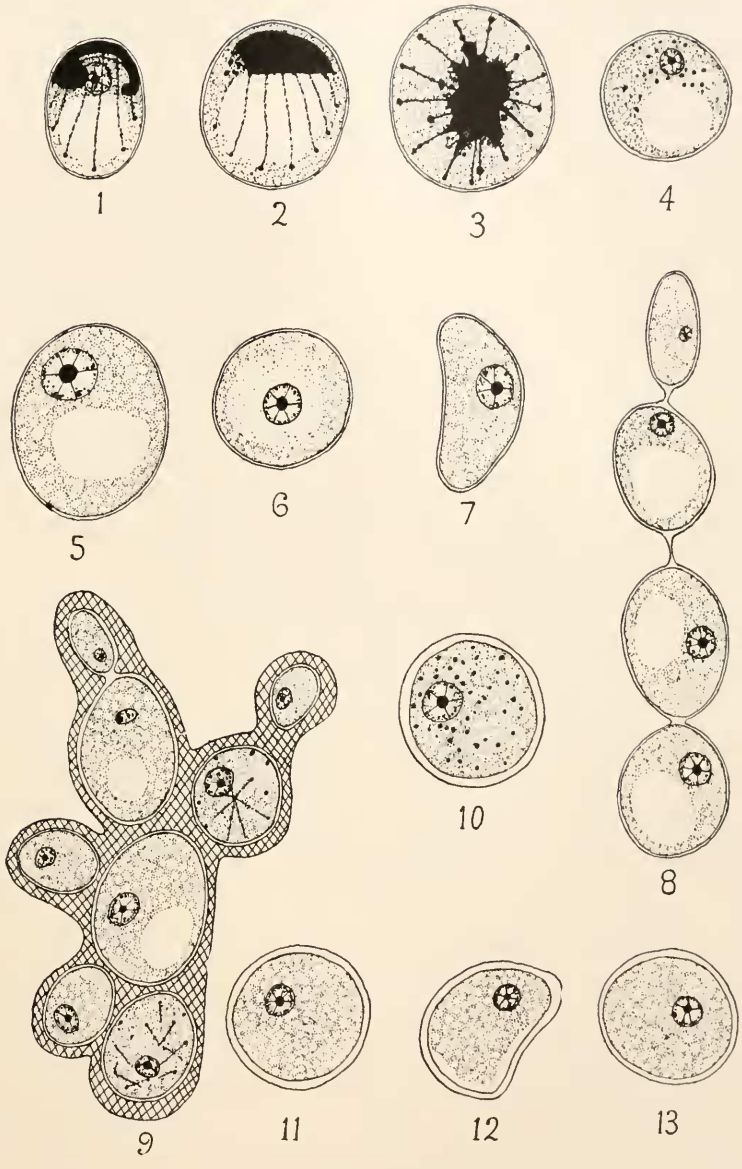

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Plate II.

Fig. 14. Early bud. Extremely large vacuole.

Fig. 15. Metachromatic granules entering the bud. Nucleus not yet beginning division.

Fig. 16. Early prophase. The chromatin is collecting on the linin strands. A few metachromatic granules visible in both cells.

Fig. 17. The nucleus is breaking up into chromosomes. Slightly oblique view making bud appear abnormally small.

Fig. 18. Metaphase. The chromosomes are separating on the equator of the spindle.

Fig. 19. Anaphase. Centrosome and spindle very evident. Metachromatic granules in both cells.

Fig. 20. The same. Slightly later.

Fig. 21. The chromosomes are passing through the isthmus. A few metachromatic granules in opposite ends of both bud and parent cell. These are much smaller than the chromosomes.

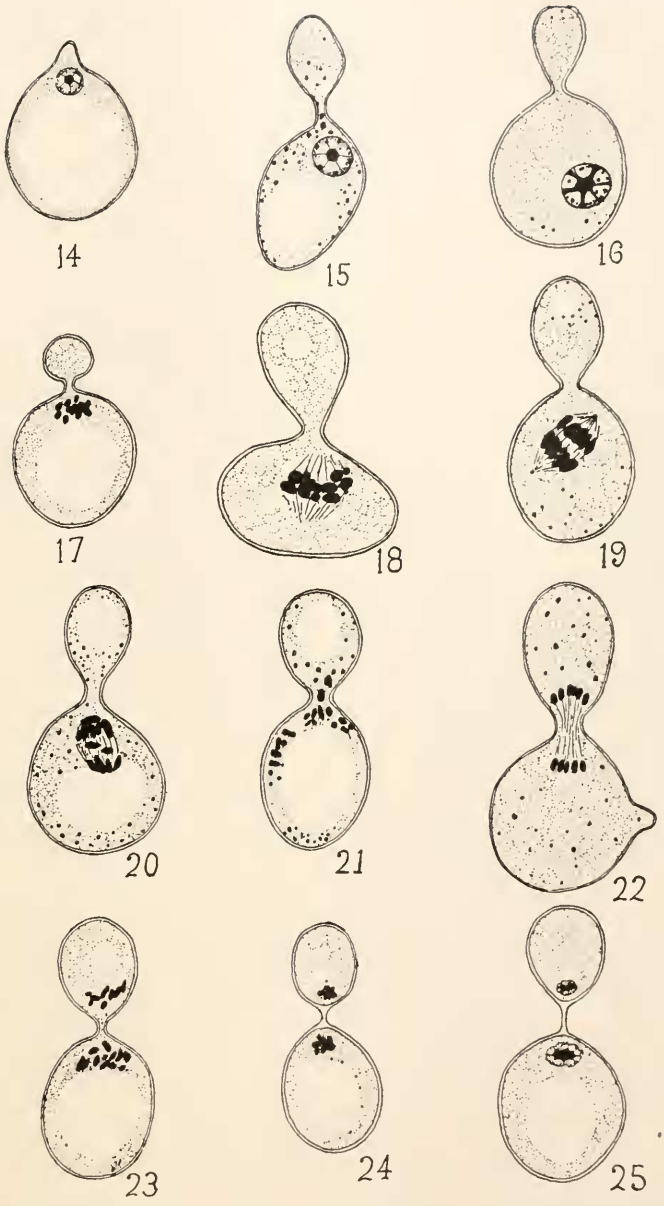
Fig. 22. Late anaphase. Spindle very evident. A second bud on the parent cell.

Fig. 23. The spindle not visible. The isthmus is closing. Chromosomes collected near one point.

Fig. 24. The cytoplasm has divided, the cells being held together by their walls. Chromosomes beginning the apparent fusion.

Fig. 25. Late telophase. Large nucleolus. Note bulges in nuclear membrane between linin strands.

Drawings by G. T. Kline.



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MEASURES OF INSECT COLD HARDINESS.

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Different measures of insect cold hardiness used by different workers may usually be reduced to the empirical survival test. Bachmetjew (1901) used the "vital temperature maximum" or the second time an insect reached the undercooling point. Duval and Portier (1922) considered that there was a freezing point below that ordinarily determined, the higher freezing point being that of the body fluids, the lower that of the body cells.

In strong contrast to the scarcity of measurements of insect cold hardiness, stand the many determinations by plant physiologists. Osmotic pressure as determined by freezing point lowering has been widely used from the time of Sachs and Pfeffer. Water content has been of value as a criterion of cold hardiness in plant groups far separated taxonomically. For example, Johnson (1923) used water content of peach buds as a measure of cold hardiness, and Steinbauer (1926) employed it for clover seeds. Newton and Gortner (1922) and Newton (1924) emphasize the importance of bound water to cold hardiness. Müller-Thurgau (1886) proved conclusively that some plants could survive freezing. The ability of a plant to survive freezing was defined by Harvey (1918) as cold hardiness.

The two kinds of insect cold hardiness (1) hardiness to the quantity factor of low temperature or ability to withstand long periods of relatively mild low temperature and (2) hardiness to the intensity factor of low temperature, or ability to withstand extremes of low temperature have been discussed in a previous paper. In the present paper cold hardiness to the intensity factor alone will be considered.

Closely associated with changes in cold hardiness are changes in moisture content. Insects dehydrated but not to the period of injury, can withstand temperatures far lower than undehydrated individuals. This is strikingly true for insects that are

not self dehydrating to any large extent. Thus the Japanese beetle, *Popillia japonica* Newm., does not exhibit any marked body weight changes over winter when kept in moist surroundings, but can be experimentally dehydrated to half its body weight. When thus treated they are very cold resistant, having a survival temperature of as low as − 28° C. In contrast

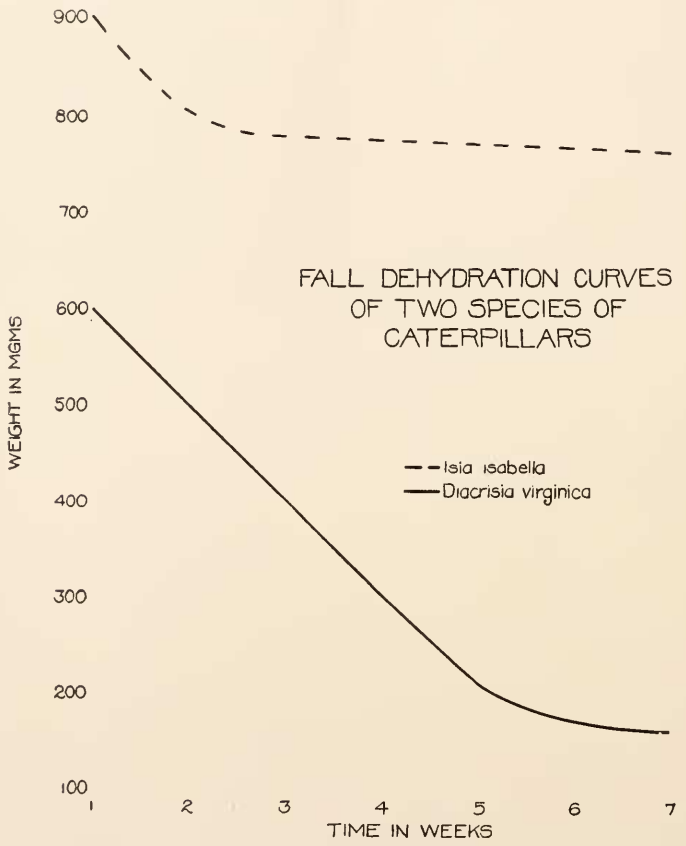

FIG. 1. Fall dehydration curves of two species of caterpillars, *Isia isabella* and *Diacrisia virginica*.

to the Japanese beetle larvæ there are some species of oak borers and caterpillars which are normally self dehydrating during the winter. The dehydration curves (Fig. 1) of *Isia isabella* Hy. Edw. and *Diacrisia virginica* Fabr. show a marked water loss as these caterpillars go into hibernation. At the period of inflection

of the weight loss curve (Fig. 1) these insects can survive freezing. When the curve is plotted with rate against weight loss the point of inflection is brought out more clearly (Fig. 2). Up to

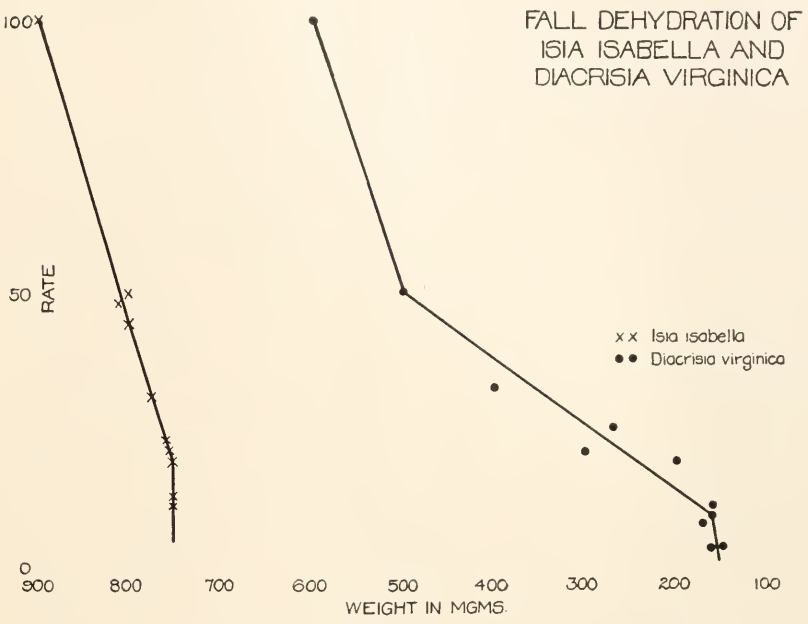

FIG. 2. Fall dehydration of *Isia isabella* and *Diacrisia virginica*. $\frac{1}{\text{time}}$ or rate plotted against weight.

the point of inflection of the weight loss curve the undercooling point of the blood is the minimum survival temperature. Beyond that point the undercooling point no longer measures the total cold hardiness which reaches to below − 40° C. There is no free body fluid on which a conductivity reading can be made.

The oak-borers, *Synchroa punctata* Neum., *Dendroides canadensis* Lec., *Romaleum rufulum* Hald. also are normally self dehydrating but never to the extent of losing all their free water. Although very cold resistant, having survival temperatures of below − 40° C., at no time even in the deepest winter, is it impossible to obtain blood samples. But conductivity is found to be proportional to the survival temperature (Fig. 3). The water content of these insects, obtained by heating them in an oven for four hours at + 50° C., is only relative but does appear

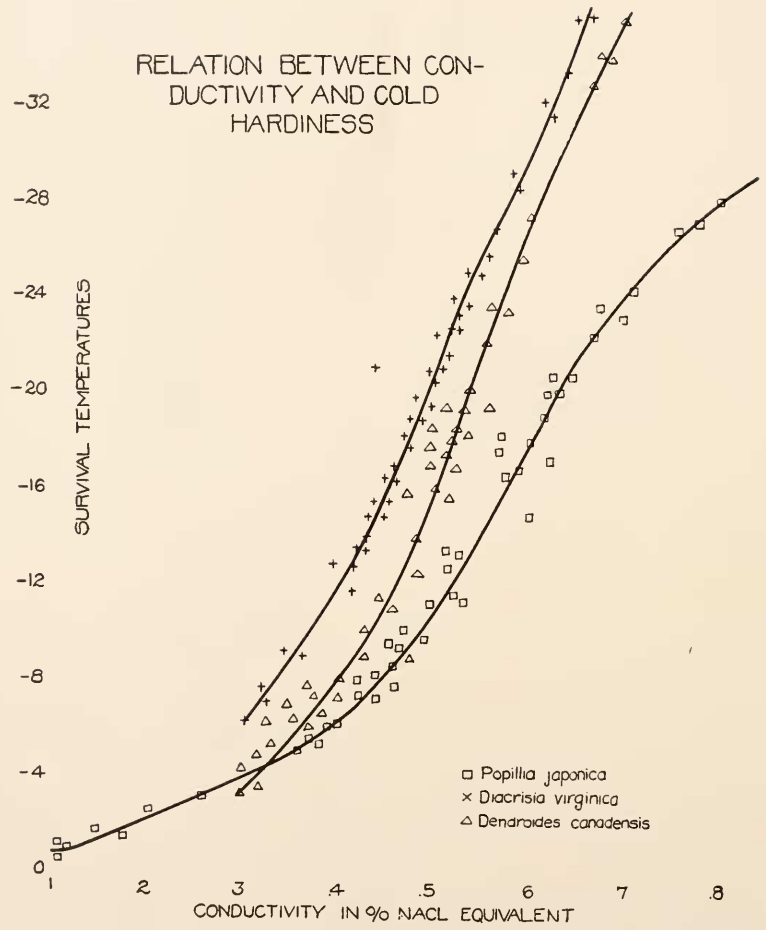

FIG. 3. Relation between conductivity and cold hardiness. *Popillia japonica* □, *Diacrisia virginica* ×, *Dendroides canadensis* △.

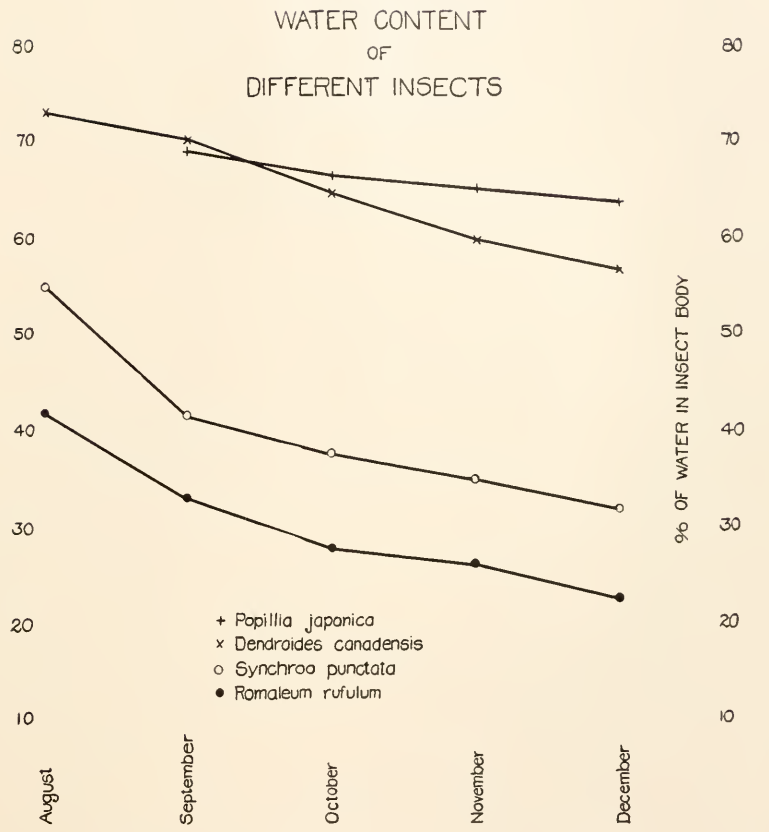

FIG. 4. Water content of different insects during the season at which they develop cold hardiness. *Popillia japonica* +, *Dendroides canadensis* ×, *Synchroa punctata* o, *Romaleum rufulum* ●.

to give comparable results with different species. The per cent. of water before and during hibernation of three species of oak-borers and of the Japanese beetle are shown in Fig. 4.

The Japanese beetle larvæ, *Popillia japonica* Neum. represent an ecological group far more protected than either the oak-borers or the woolly bear caterpillars. This species hibernates in the ground below the frost line. About 97 per cent. are third instar larvæ and about 3 per cent. second instar. There is a cyclic change in the cold hardiness of these larvæ, not as marked, however, as in the oak-borers but more apparent than in the

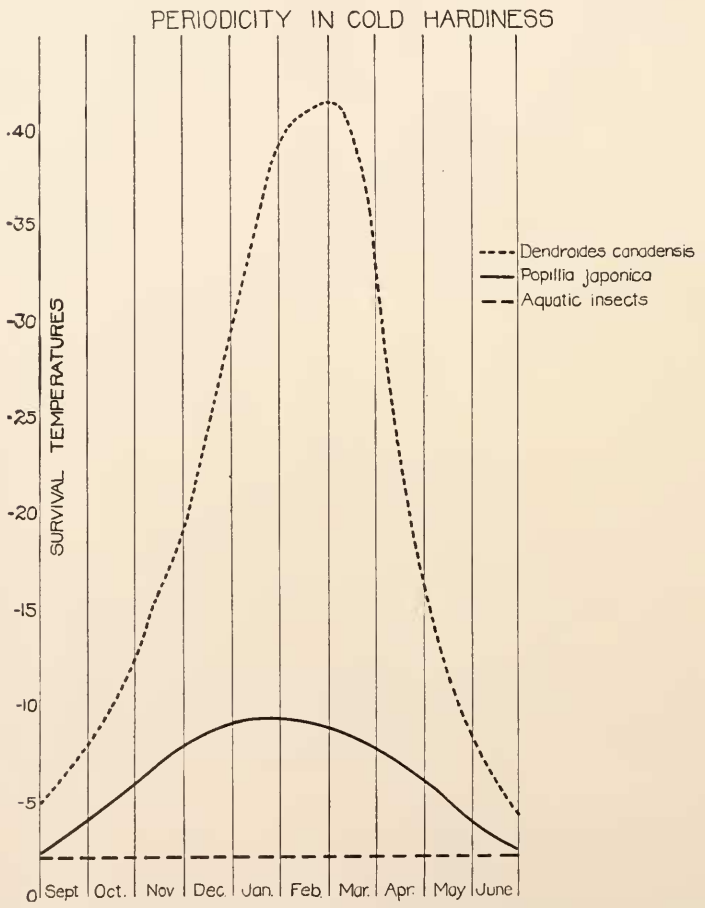

FIG. 5. Periodicity in cold hardiness. *Dendroides canadensis* – – –, *Popillia japonica* ——, aquatic insects — —.

aquatic insects where there is practically none, Payne (1926). This periodicity in cold hardiness is shown in Fig. 5. The relation between undercooling and survival temperatures is shown in Fig. 6. Cold hardiness greater than is usually found in their

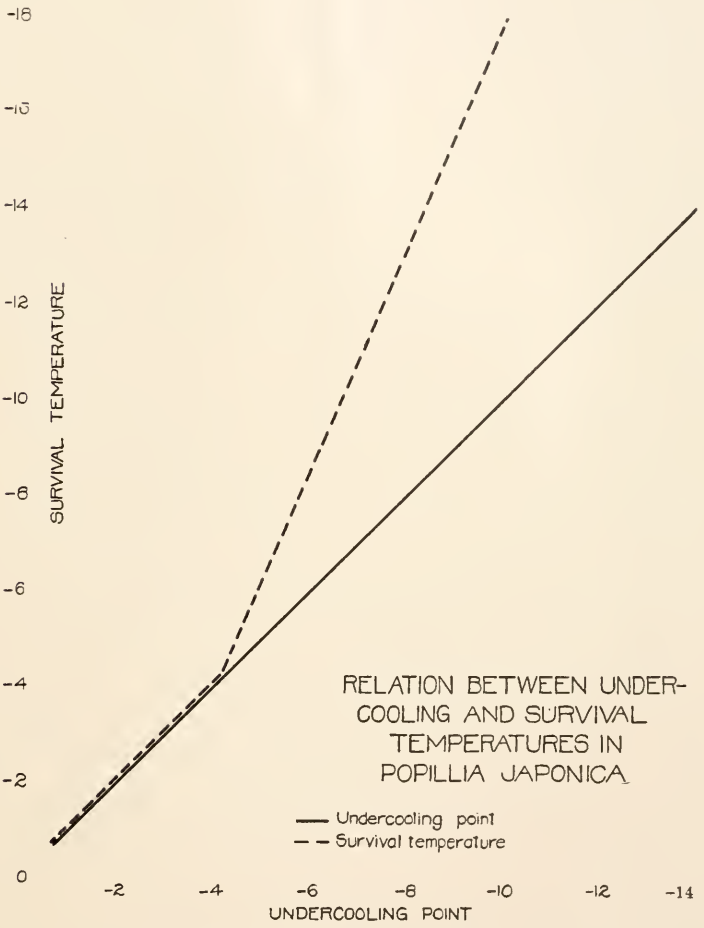

FIG. 6. Relation between undercooling and survival temperatures in *Popillia japonica*. Undercooling point ——; survival temperature — —.

soil habitat can be induced in this insect by dehydration. Conductivity measurements of the blood of dehydrated insects were made. The greatest cold hardiness was found in the dehydrated insects and the least in those infected with wilt disease or

polyhedrälskrankheit. In this disease both the freezing point and the conductivity of the blood approach that of water. In Fig. 3 the conductivities of the Japanese beetle larval blood are plotted against survival temperature. Cold hardiness in this species is more fully measured by conductivity than by either moisture content or undercooling point.

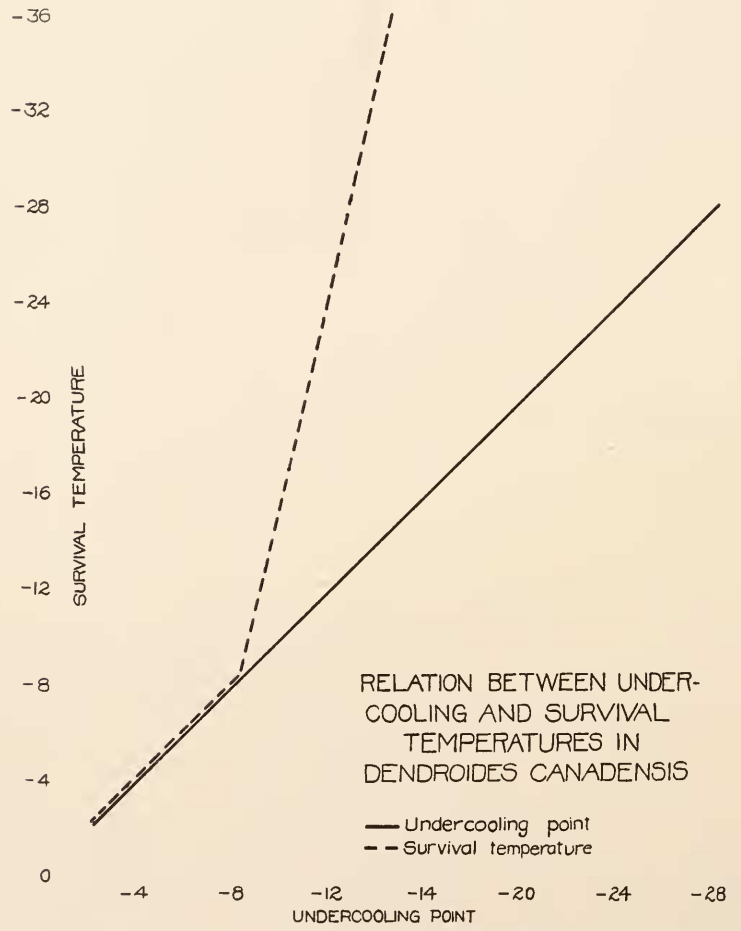

FIG. 7. Relation between undercooling and survival temperatures in *Dendroides canadensis*. Undercooling point ——; survival temperature — —.

Summary.

1. Cold hardiness to the intensity factor of low temperature can be measured by moisture content, undercooling point, and blood conductivity.

2. Up to the time when a given insect can survive freezing, undercooling is a reliable measure of cold hardiness. Beyond the point when an insect can survive freezing, undercooling measures but a part of the total cold resistance of a given insect.

3. Conductivity measurements are found proportional to cold hardiness throughout the whole year. In some insects there is insufficient free body fluid in winter on which to determine blood conductivity.

4. For each species there is a different set of physical constants which measure the cold hardiness of that species.

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COMPENSATORY HYPERTROPHY OF THE TESTES IN BROWN LEGHORNS.[1]

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I. Introduction.

Compensatory hypertrophy of the surviving testis after unilateral castration was observed as early as 1890 by Ribbert (1). Ribbert worked on mammalian material, using young but almost mature rabbits. Ribbert removed the right or the left testis and then compared the surviving gland with controls of the same age after three months; considerable hypertrophy was almost always found. The hypertrophied testis weighed six times as much as one control gland in three cases. There was no difference in the degree of hypertrophy between the right or the left testis. If the operated animal did not increase in weight as much as did the normal controls, then the retained gland also showed a corresponding lack of development.

Lipschütz '22 (2) repeated these experiments of Ribbert, using also rabbit material for his experiments. It appears to follow from Lipschütz's paper that there is a compensatory increase in weight of the surviving testis after unilateral castration when the operation is performed on young rabbits. This increase in size and weight becomes progressively less the longer the operated animals are kept; about one year after the operation there is not much difference in the weight of the surviving testis and in that of one of the control pair of glands. Unilateral castration is not followed by a significant increase in the size of the remaining testis when the gonad is removed in adult rabbits. Lipschütz believes that his results indicate that the testis is incapable of true compensatory hypertrophy; the actual increase in weight observed being due only to a more rapid rate of growth of the isolated gonad.

[1] From the Whitman Laboratory of Experimental Zoölogy of The University of Chicago. The expenses of this investigation were supported in part by the Committee for Research in Problems of Sex of the National Research Council; grant administered by F. R. Lillie.

The two papers cited above report a certain difference in the results obtained. The discrepancy may be due to the time the surviving gonad was retained and also to the age of the animal at the time of operation.

We became interested in the problem of compensatory hypertrophy of the testis as applied to the material used in the laboratory for a variety of experiments, pure bred Brown Leghorn cocks. The experiments were begun early in July 1924 and terminated at the end of April 1925.

We were guided in outlining the course of the experiments by the following points of view: (*a*) the appearance of compensatory hypertrophy as such after the removal of one of the pair of gonads; (*b*) the influence of the age of the birds at the time of the operation on the possible increase in size of the surviving gonad; (*c*) whether the time that the surviving gonad is retained is of effect on the degree of possible hypertrophy taking place. (*d*) Finally we wished to determine if there was a significant difference in the amount of the hypertrophy taking place in the right or the left gonad after unilateral castration.

After the experiments were terminated and the data completed we became acquainted with a paper by Benoît '25 (3). This author carried out a series of unilateral castrations on White Leghorns; the operations were performed on three young birds of 18–20 days of age, on one young bird aged two months and on two birds aged seven months each. Control gonad weights were stated for the groups of different ages. According to the results obtained by Benoît, there is a very real increase in the weight of the surviving gonad when castration is carried out at an early date. In the three cases where unilateral castration was performed in baby chicks the surviving testes were retained for about twelve months. At this date each one of the hypertrophied testes weighed approximately as much as, or slightly more than, both testes of the control. The surviving gonad of the cockerel which was operated upon at two months was retained for seven months, at the end of this time it weighed almost 50 per cent. more than the control pair of testes, but it is important to observe that "controls" of this age vary greatly among themselves. Benoît observed no significant hypertrophy

when unilateral castration was performed on birds aged seven months. The surviving testis was retained about a year. Benoît concludes that there is a hypertrophy of the surviving gonad when the one member of the pair is removed in very young birds; unilateral castration of older birds, after the testes have achieved approximately their normal size is not followed by a compensatory increase in the weight of the surviving gonad.

The results published by Benoît agree on the whole with those obtained in this laboratory, but we differ slightly from him in the observations on older birds as will appear in the discussion.

The phrase "compensatory hypertrophy" defines the conception, viz: that loss results in stimulating the growth of the surviving member to an extent that tends to restore a normal quantitative balance between the total gonad tissue and the bird. It involves the corollary that there is a normal quotient for weight of bird divided by weight of gonad tissue. The present study aims merely at testing this assumption. The difficulties arise from the fact that the assumed normal quotient of weight of bird divided by weight of gonad varies (1) with age very markedly; (2) with the time of year, age being the same; and that (3) no organ of the body probably is so susceptible to general conditions of health as the testis. These difficulties create numerous sources of error for any very exact formulation, so that we felt that it was not desirable in the present status of this subject with reference to our main problems to use a sufficient amount of material and time to reach quantitative results. The present study, although it gives positive results, is therefore merely suggestive.

It is a pleasure to express our thanks to Professor F. R. Lillie for his continued interest in the work and for his helpful suggestions during its course.

II. Data on Unilateral Castration.

All the unilateral castrations were carried out on pure bred Brown Leghorn cockerels that were obtained from one well-known source. The birds were divided into four groups, the first being about one week of age, the second sixteen weeks, the third

twenty-four weeks and the fourth between thirty-two and forty weeks of age at the time of operation.

The operated birds and their controls in each group were hatched at the same time and kept in the laboratory under identical conditions. Comparisons were made only within the groups and in no case between birds of the same age but hatched at different periods.

The cockerels and their controls were weighed at the time of operation and the measurements of the head furnishings and spurs taken. These observations were repeated every eight weeks and a record kept of the condition of the experimental and control birds during the entire experimental period. The testes were removed through an incision between the last two ribs, the gland rapidly weighed and the volume obtained through displacement of normal saline. In the group of baby chicks where the testes were removed at about one week of age, the gonads were not weighed, but the length and width of the gland taken with a pair of fine callipers. The removed testes were fixed in Bouin's fluid at 37° C. and kept in the incubator at that temperature for several hours, varying with the size of the gonad. The usual procedure was followed in washing, etc., and the testes preserved in oil of wintergreen for future histological work.

The greater number of the chicks operated upon at one week of age was lost together with their controls owing to unfavourable weather conditions. The survivors were kept until they were thirty-two weeks of age and then completely caponized or killed.

In the three other groups the right gonad was removed from a certain number of cockerels and the left from a similar number. The surviving right or left testis was then retained for eight, sixteen and twenty-four weeks respectively, at the end of each of these periods one cockerel having a right testis, one cockerel having a left testis and two control birds were completely caponized or killed.

We found in the course of our observations that loss of weight on the part of the cock is reflected in a corresponding diminution of the size of the testes.

The data obtained are outlined in the tables given below. The age of the bird at the time of the operation, the weight of

the bird at the beginning and end of the experimental period is stated. The weights of the removed, hypertrophied and control glands are expressed in grammes and in per cent. of the body weight of the fowl.

In the group A (Table I.), where unilateral castrations were performed on baby chicks aged one week, six operated birds and

Table I.

Results of Unilateral Castration at One Week.

No.	Observations at Time of Operation.			Weighings at 32 Weeks.		
	Chick, Weight.	Removed Testis.		Bird.	Surviving Testis.	
		Length.	Width.		Weight.	Per Cent. Weight.
53.....	45.40	R. 3.5	2.0	1,460.80	L. 19.95	1.35
56.....	54.50	L. 4.1	1.2	1,507.50	R. 7.35	0.48
44.....	67.15	R. 4.5	2.0	1,545.85	L. 13.00	0.83
49.....	66.00	L. 5.0	1.3	992.25	R. 0.85	0.095
46.....	57.95	R. 5.5	1.5	1,275.85	L. 18.70	1.46
50.....	50.90	L. 6.0	1.0	1,048.96	R. 5.10	0.49
62c[1].....	45.00			1,020.60	R. 0.65	0.063
					L. 0.55	0.053
64c.....	46.50			1,602.55	R. 12.80	0.79
					L. 11.25	0.70
65c.....	47.65			1,219.05	R. 6.95	0.57
					L. 7.50	0.61
63c.....	43.00			1,489.15	R. 8.20	0.55
					L. 7.75	0.52
66c.....	44.00			994.25	R. 6.20	0.61
					L. 5.85	0.58

The measurements given for the testes removed at unilateral castration are in mm. All weights are in grammes. Per cent. weight = percentage of weight of testis to total weight of bird.

[1] c = control.

five controls survived for a period of about thirty-one weeks. Unilateral castrations were performed on thirty-five baby chicks and there were a large number of controls; the mortality was due not so much to operative effects but to the very unfavourable weather conditions. Of the six surviving operated birds, three had a left testis while the other three had a right one. Each surviving left gonad is larger than any of the ten control testes; in two of the three cases each surviving left testis is larger than

any of the five control pairs with one slight exception (No.64). These results cannot be due to chance; we are therefore justified in concluding that a surviving left testis, the partner of which is removed at one week after hatching, exhibits a much greater amount of growth than it would have done, approaching in some cases twice the normal growth.

Preliminary histological examinations of sections of hypertrophied testes indicate that all the gonad tissues are equally concerned in this increase in size.

TABLE II.

RESULTS OF UNILATERAL CASTRATION AT 16 WEEKS.

No.	Weighings at Time of Operation.			Later Observations.		
	Bird.	Removed Testis.		Bird.	At 24 Weeks.	
					Surviving Testis.	
		Weight.	Per Cent. Weight.		Weight.	Per Cent. Weight.
819.....	992.25	R. 0.20	0.020	1,687.60	L. 9.60	0.56
820.....	1,077.35	L. 0.35	0.031	1,630.90	R. 6.35	0.38
826c[1]....	1,105.65			1,630.90	R. 8.40	0.50
					L. 9.80	0.60
830c.....	1,162.35			1,602.55	R. 3.00	0.18
					L. 3.10	0.19
					At 32 Weeks.	
815.....	1,048.95	R. 0.36	0.033	1,687.60	L. 30.00	1.81
823.....	907.20	L. 0.17	0.018	1,247.40	R. 15.82	1.26
829c.....	1,460.80			2,071.10	R. 14.11	0.66
					L. 15.78	0.75
831c.....	1,048.95			1,857.70	R. 11.82	0.60
					L. 14.11	0.75
					At 40 Weeks.	
824.....	1,048.95	L. 0.65	0.061	1,574.20	R. 20.18	1.28
825.....	963.90	L. 0.19	0.19	1,517.50	R. 20.25	1.31
833c.....	992.25			1,574.20	R. 11.61	0.73
					L. 14.30	0.90

All weights are in grammes. Per cent. weight = percentage of weight of testis to total weight of bird.

[1] c = control.

The three surviving right testes on the other hand showed no such increase in size, and in fact did not differ significantly from a single control testis.

The second group of young cockerels was castrated at sixteen weeks and the results are tabulated in Table II. The surviving testes were retained eight, sixteen and twenty-four weeks respectively. It was originally planned to observe the degree of compensatory hypertrophy to forty-eight weeks at which time the birds have been fully mature for about sixteen weeks. However a number of birds died owing to one cause and another so that the last data were obtained on cocks aged forty weeks and only on two right testes.

There is no observable compensatory hypertrophy either of the right or the left testis when the glands were retained only for eight weeks after the operation. There was an increase in size during this period but this was identical with the control glands. After a period of sixteen weeks, however, both the left and the right surviving testes show a considerable degree of compensatory hypertrophy, the left testis being heavier than both testes together of each of the two control pairs; the right testis on the other hand, while it exhibited a high percentage weight, was only slightly heavier absolutely than a single control testis. Three birds were available for observation after twenty-four weeks, two of them having a right testis each while the third served as control. Each of the two right testes weighed about 75 per cent. as much as the control pair, the degree of compensatory hypertrophy on a percentage basis being similar to the amount observed after sixteen weeks.

Table III. gives the data for the next group. The cockerels in this group were unilaterally castrated at twenty-four weeks and then observed to forty-eight weeks at intervals of eight, sixteen and twenty-four weeks, respectively. After eight weeks the surviving left testis showed a certain degree of hypertrophy, weighing much more than one of the control pair of gonads and only slightly less than the other. The right testis had not increased at all as compared with the normal. The same is true after sixteen weeks; the left surviving gland is larger than any one testis of the control pairs but not as heavy as one of the

Table III.

Results of Unilateral Castration at 24 Weeks.

No.	Weighings at Time of Operation.			Later Observations.		
	Bird.	Removed Testis.		Bird.	At 32 Weeks.	
					Surviving Testis.	
		Weight.	Per Cent. Weight.		Weight.	Per Cent. Weight.
810	1,574.20	R. 0.45	0.027	1,801.00	L. 10.90	0.60
801	1,332.45	L. 0.20	0.016	1,715.95	R. 0.65	0.041
901c[1]				1,574.20	R. 1.50	0.095
					L. 1.31	0.093
902c				2,241.20	R. 6.65	0.29
					L. 8.95	0.39
					At 40 Weeks.	
813	1,020.60	R. 0.25	0.024	2,127.80	L. 17.05	0.80
804	1,545.85	L. 5.0	0.32	2,241.20	R. 8.60	0.37
809c				2,212.85	R. 12.70	0.57
					L. 14.60	0.65
907c				2,099.45	R. 7.70	0.36
					L. 8.0	0.38
					At 48 Weeks.	
808[2]	963.90	R. 0.20	0.020	1,162.35	L. 3.18	0.27
812	1,573.20	L. 2.70	0.17	1,212.85	R. 10.83	0.83
904c				2,127.80	R. 13.23	0.62
					L. 11.81	0.55
905c				2,042.75	R. 4.15	0.20
					L. 3.45	0.11

All weights are in grammes. Per cent. weight = percentage of weight of testis to total weight of bird.

[1] c = control.

[2] No. 808 was ill during the first half of the experimental period and lost considerable weight which it had not regained at the time the bird was killed and the surviving testis removed.

pairs, while the right surviving testis is not as heavy as control single testes. After twenty-four weeks there was no hypertrophy at all to be observed in the left surviving testis; as this bird had been in poor condition and lost considerable weight during the experimental period, we do not attach much significance to

this case. The right surviving testis is probably to be regarded as hypertrophied, being heavier than one of the control pairs and its percentage weight greater than any one testis of the other control pair.

In the group where the cocks were mature (Table IV.), there was not so much difference to be observed in the hypertrophy of the surviving testes. The left testis had increased relatively more in size compared with the gland removed at the operation than the right testis. The hypertrophying testes were retained

Table IV.

Results of Unilateral Castration at 32–40 Weeks.

No.	Weighings at Time of Operation.			Weighings at 40–48 Weeks.		
	Bird.	Removed Testis.		Bird.	Surviving Testis.	
		Weight.	Per Cent. Weight.		Weight.	Per Cent. Weight.
121.....	1,574.20	R. 3.95	0.25	2,042.75	L. 13.87	0.67
114.....	1,517.50	L. 6.55	0.43	1,659.25	R. 14.27	0.86
123c[1]....	1,517.50			1,517.50	R. 7.36	0.48
					L. 7.52	0.49
128c.....	1,829.35			1,687.60	R. 5.0	0.39
					L. 4.93	0.39

All weights are in grammes. Per cent. weight = percentage of weight of testis to total weight of bird.

[1] c = control.

for only eight weeks, the observation period being planned to extend only to forty-eight weeks of age. The left surviving testis was larger than any single testis of the two control pairs, but not as heavy as either pair together. The right surviving testis was heavier than one control pair and almost equal to the other pair, being 0.86 per cent. of the body weight while the two control testes pairs weighed 0.78 per cent. and 0.97 per cent. respectively. While the cases are few, the evidence seems to indicate compensatory hypertrophy in this group also after a very short period.

IIa. Summary of Results of Unilateral Castration at Various Ages.

The preceding tables and descriptions demonstrate that removal of one of the testes pair induces an increase in size of the retained partner when the retention period is of sufficient length, with the exception of the right isolated testes of young chicks (Table I.).

When unilateral castration is performed on young cockerels the left surviving gonad hypertrophies to a greater degree than does the right one, the one differing result obtained in No. 808 being due to the bird's general condition.

The increase in weight of the isolated testis becomes manifest only after a certain interval following castration, the interval being apparently somewhat longer for the right hypertrophying testis than for the left one when younger birds are operated upon.

Table V.

Data Compiled from Tables I.–IV. Control Testes Weights Stated as Averages.

Age of Bird.		Weight of Isolated Testis.	Average Weight of Controls.	
At Operation.	At Removal.		Testis Pair.	Single Gland.
One week.....	32 weeks	L. 13.00 L. 18.70 L. 19.95 R. 0.85 R. 5.10 R. 7.35	13.54 (5p.)	6.77
16 weeks.....	24 weeks	L. 9.60 R. 6.35	12.15 (2p.)	6.07
	32 "	L. 30.00 R. 15.82	27.91 (2p.)	13.98
	40 "	R. 20.18 R. 20.25	25.91 (1p.)	12.85
24 weeks.....	32 weeks	L. 10.90 R. 0.65	9.10 (2p.)	4.80
	40 "	L. 17.05 R. 8.60	21.50 (2p.)	10.75
	48 "	L. 3.18 R. 10.83	16.32 (2p.)	8.16
32–40 weeks.....	40–48 weeks	L. 13.87 R. 14.27	12.40 (2p.)	6.20

All weights are in grammes.

The latent period is much shorter when adult birds are unilaterally castrated and here the right isolated testis hypertrophies at the same rate and in the single case available even to a slightly greater degree than the left (Table IV.).

Comparison with the averages of the controls rather than with single controls as set forth in the tables increases the probability of these conclusions as examination of Table V. shows. Individual cases emphasize these general conclusions (Table I., Nos. 53, 44, 46. Table II., Nos. 815, 824, 845. Table IV., Nos. 114, 121).

Lipschütz's suggestion for mammals that unilateral castration produces only a more rapid rate of growth rather than a definitive compensatory enlargement of the surviving gonad does not seem a probable interpretation of our results. It would mean that there would be no actual plus in weight of the isolated gonad over one of the control testes pair at the end of the developmental period of the glands. Such a statement requires a definition of the endpoint of growth of the testes, and in view of the normal variation in the weight of the testes as well as the seasonal variation the feasibility of such an absolute determination appears questionable.

The cocks are mature at thirty-two weeks and compensatory hypertrophy as defined is demonstrated not only at this time but as late as forty-eight weeks.

III. Normal Size Relations of Right and Left Testes.

During the course of the experiments we accumulated some data on the size of the right and the left testes of normal cocks. The majority of the observations were made on the gonads of different birds but the records of the control birds in the preceding tables are for pairs. Tables VI. and VII. give the measurements obtained in one week old chicks. The length and width of the right and left testes are given as it was impracticable to secure accurate weights. The weights of the chicks are also stated for comparison. There appears to be a very slight advantage in size on the part of the left testes at this age. In older birds we find such an individual variation occurring in birds of the same age and even of approximately

TABLE VI.

MEASUREMENTS OF LEFT TESTES REMOVED FROM CHICKS AGED ONE WEEK.

No.	Testes.		Weight of Chick.
	Length.	Width.	
51	4.4 mm.	1.5 mm.	45.15 gs.
61	5.1 "	2.0 "	55.30 "
62	4.0 "	1.8 "	45.0 "
0	3.5 "	1.1 "	39.30 "
63	5.0 "	1.2 "	43.0 "
64	4.5 "	2.0 "	46.50 "
65	3.0 "	2.0 "	47.65 "
56	4.1 "	1.2 "	54.50 "
64A	4.0 "	1.1 "	40.40 "
66	4.0 "	1.5 "	44.0 "
67	5.0 "	1.5 "	49.50 "
68	5.0 "	1.9 "	44.75 "
69	4.8 "	1.9 "	49.90 "
50	6.0 "	1.0 "	50.90 "
49	5.0 "	1.3 "	66.0 "
48	5.3 "	1.3 "	57.85 "
40	4.5 "	1.5 "	52.80 "
47	5.2 "	1.7 "	57.85 "
39	5.0 "	1.3 "	63.80 "

No. of Cases.	Average of Measurements of Testes. Length.	Width.
19	4.6 mm.	1.5 mm.

TABLE VII.

MEASUREMENTS OF RIGHT TESTES REMOVED FROM CHICKS AGED ONE WEEK.

No.	Testes.		Weight of Chick.
	Length.	Width.	
52	3.5 mm.	1.0 mm.	43.75 gs.
54	4.3 "	1.1 "	43.87 "
53	3.5 "	2.0 "	45.40 "
55	3.0 "	1.5 "	42.60 "
57	5.0 "	1.5 "	49.0 "
58	4.0 "	1.0 "	43.30 "
59	4.0 "	1.3 "	45.60 "
61	5.1 "	2.0 "	55.30 "
70	4.0 "	1.5 "	39.90 "
46	5.5 "	1.5 "	57.95 "
44	4.5 "	2.0 "	67.15 "
43	6.7 "	1.5 "	56.30 "
42	6.0 "	1.5 "	51.10 "
41	5.0 "	1.5 "	59.0 "

No. of Cases.	Average of Measurements of Testes. Length.	Width.
15	4.5 mm.	1.2 mm.

identical weight, that valid conclusions cannot be drawn from the data obtained in different cocks. The tables compiled are omitted for this reason.

Where testes of one pair were observed as was done for the control cockerels (Tables II.–IV.) the left testes were larger than the right in one case out of two at twenty-four weeks. At thirty-two weeks, the left testes were larger in two out of three pairs, while at forty-eight weeks, the left testis was very slightly heavier (0.01 per cent.) than the right one in one pair; it was smaller than the right testis in two pairs and finally there was one pair in which the gonad weighed exactly the same amount on the left and on the right side.

The tendency of the left testis to be rather larger than the right one in embryonic chicks has been observed by a number of authors. Firket, '14 (4), states that the right testis is noticeably smaller than the left one in the chick at the seventh day of incubation and quotes Semon, '87 (5), as saying that the left testis is much larger at the beginning of its development.

According to Swift, '16 (6), the left embryonic testis is noticeably larger than the right one in the five day chick and the germinal epithelium of the left gonad is also thicker and more extensive. This difference in favor of the left testis is also visible in the six and nine day chick. Riddle, '16 (7), finds no difference between the right and the left testes in common fowl, the age of the birds is not stated.

The greater tendency towards hypertrophy of the left testis discussed under II.*a*, is presumably associated with this embryonic condition, and is of interest in comparison with the very pronounced asymmetry of the female.

IV. Discussion.

From the results described in the preceding pages as well as from the experimental data published by Benoît, the occurrence of compensatory hypertrophy following unilateral castration in young male fowls seems to be well established. We found compensatory hypertrophy of the retained gonad also in adult cocks, differing in this point from Benoît's observations. The period during which the surviving testes were permitted to

hypertrophy was shorter in our cases than in those reported by Benoît and it is possible that the explanation for the different results obtained may be found in this fact.

The problem of compensatory hypertrophy of the gonad presents its teleological as well as its physiological aspects. The term itself has teleological implications; from this point of view the "purpose" might be either to provide increased reproductive capacity, which appears unnecessary, or to establish a balance of hormones. There again the solution is unsatisfactory for less than one testis is sufficient for maintenance of sex characters as shown by Pézard, '21 (8); '25 (9); Champy, '25 (10).

Physiologically considered it would appear to be obvious that the growth of testis tissue is balanced against something else in the organism.

The general bodily metabolism favors the growth of a definite amount of gonad tissue and no more. The removal of one testis of a pair leaves a balance of conditions favorable to the continued growth beyond its normal size of the surviving member which thereupon responds in proportion to its growth capacity up to the limits of the favorable metabolism. When unilateral castration is performed very early this may result in a single testis greater in weight than a normal pair (Table I., case 16, Table II., case 815), whether there may be a progressive limitation of capacity for compensatory growth with increasing age as maintained by Benoît is still an open question as far as our own results are concerned.

No theory is put forth in explanation of the change in the reaction between gonad and organism which follows removal of one of the gonad pair; it is shown by the facts. But the importance of the principle appears again in the transformations of the female following ovariotomy. The right rudimentary gonad responds with a proliferation of the kind of tissue of which it is composed at the time the demand on it is created, thus producing the various types of right compensatory growth described in completely and incompletely castrated hens by Domm, '24 (11); '27 (12). The principle of compensatory hypertrophy is also illustrated in the growth of grafts.

V. Summary.

1. Unilateral castration in Brown Leghorn cockerels is followed by compensatory hypertrophy of either the right or the left retained testis when the operation is performed on birds aged 16, 24 and 32–40 weeks.

2. The removal of the right or the left testis in chicks aged one week caused a compensatory hypertrophy of the left retained gonads only, after a period of thirty-one weeks, in our experiments (Table I.).

3. There may be a certain period before increase in weight of the retained gonad over the controls becomes manifest; the length of this period is variable.

4. The left retained testis shows a greater tendency towards hypertrophy than does the right.

5. There seems to be a difference in the weight of the left and the right normal testes; this is in favor of the left gonad in very young birds and then gradually seems to become shifted to the right testis as the bird becomes older and reaches maturity.

6. A tentative suggestion is made, that there may be some relation between the greater amount of germinal epithelium in embryonic left testes and the greater tendency towards hypertrophy of the left surviving gonad which is particularly manifest when unilateral castration is performed on young birds.

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NOTE ON THE HEMOLYTIC ACTION OF SEBRIGHT SERUM ON LEGHORN CORPUSCLES.[1]

MARY JUHN.

In a series of experiments on grafts of Leghorn testes into Sebright capons and Sebright testes into Leghorn capons carried out by Mr. Roxas in this laboratory (1), the observations were made that the Leghorn testis takes readily in the Sebright but that the inverse is not the case. A much greater mortality was also observed in the Leghorn hosts having Sebright grafts than in the Sebrights having Leghorn grafts. In actual figures:

	Leghorn Testis into Sebrights.	Sebright Testis into Leghorns.
No. of birds	38	50
Died from known causes	12, 31%	8, 6%
Died, cause unknown	8, 21%	24, 48%
Survived to end of exp.	18, 47%	18, 36%
Birds with succ. grafts (% of surviving birds)	11, 60%	3, 8%

According to a verbal communication by Mr. Roxas, the Leghorn capons with Sebright grafts that succumbed, showed evidences of pronounced anæmia, this being evident in the lack of color of the headfurnishings which became progressively almost a dead white.

In attempting to determine the causes for the greater mortality among the Leghorn hosts as well as the reason for the much smaller per cent. of successful grafts of Sebright testes into the Leghorns, the following suggestions present themselves. Considering first the conditions in the Sebrights having Leghorn testes grafts, the per cent. of takes is high; the mortality which may be attributed to the graft (cause of death unknown, 21 per cent.) is relatively not so pronounced. We may believe then that the Leghorn tissues are readily incorporated in the

[1] From the Whitman Laboratory of Experimental Zoölogy of the University of Chicago. The expenses of these investigations were supported in part by the Committee for Research in Problems of Sex of the National Research Council; grant administered by F. R. Lillie.

Sebright organism, they soon become vascularized, and being supplied with nutritive substances, persist and even show active growth. There is no extended necrosis in the Leghorn grafts beyond some presumably occurring when it is first implanted and previous to vascularization.

When Sebright testes are implanted into Leghorn tissues however, there is a relatively high percentage of mortality attributable to the graft (cause of death unknown, 48 per cent.) and a very low percentage of takes, 8 per cent. of the surviving birds and only 6 per cent. of all the birds operated upon. There is no evidence of vascularization of the Sebright testis grafts, on the contrary the appearance of the Leghorn capons after Sebright implantations have been made lead one to believe in a greater activity of the lymphocytes and a subsequent elimination of the destroyed graft tissues into the blood stream of the host. Blood counts before and after grafting as well as control of the body temperature would presumably serve to determine the accuracy of these assumptions. The resorption of the graft in a large percentage of cases with subsequent mortality of the host from one cause or another is however apparent.

There is an extensive literature on the subject of the toxicity of organ extracts when injected intraperitoneally, subcutaneously or intravenously. The intravenous injections produce the most rapid lethal effects, but subcutaneous injections of organ paste into guinea pigs, were reported by Brieger and Ulenhuth (2) to kill the animals within 24 hours after the injection. This effect was correlated by Dold and Kodama (3) with the toxic action of tissues in a state of destruction and with the causes of death after burns. Pfeiffer (4) states that in acute cases of death from burns, the cause of the mortality may be traced to toxic poisoning induced by protein fission products. These products appear in excessive quantities owing to the resorptive destruction of the proteins which have been changed and killed through heat.

It appears reasonable in view of the findings in comparable fields reported above, to correlate the percentage of deaths in the Leghorns having Sebright grafts with the non-success of these grafts. The continuous resorption of the graft tissues, may, and probably does, set free into the blood stream of the

host toxic substances resulting from the destruction of the implant, and these toxic substances finally prove fatal to the carrier of the graft.

The point of interest is the reason for the different results obtained in these cross-transplantations. The possibility of some specific differences in the blood of the two breeds of fowl was suggested by Prof. F. R. Lillie; the tests made to establish the presence of such differences are described in the latter part of the paper.

My thanks are due to Professor Lillie both for suggesting the problem reported here and for his continued helpful interest in the work.

Some experiments carried out by Sokoloff (5) serve to illustrate that such conditions can be found in other vertebrates. Sokoloff working on homotransplantations in rabbits found the presence of three types of blood and states that grafts only take when made into animals of identical blood constitution with the donor. When grafts are made into animals where the serum of the host agglutinates the corpuscles of the donor, the graft shows complete necrosis. This destruction of the graft cells leads to the production of specific antibodies in the host which have an unfavorable action on the graft. Any graft will cause the formation of some percentage of antibodies but these are counteracted by the graft as soon as vascularization is established and they then disappear from the blood stream.

Furthermore according to Sokoloff, immunization by intraperitoneal injections of an emulsion of the organ to be grafted causes the appearance of antibodies as well, and the presence of these antibodies inhibits take and growth of the graft.

In studying the conditions in the Leghorns and Sebrights, no tests were made for the determination of circulating antibodies. The sera of Leghorn and Sebright cocks and capons were tested for their agglutinating and hemolytic action on the corpuscles of all four kinds of birds.

The experiments were repeated four times, the preparation of the serum and the corpuscle suspension being identical in every case. For the corpuscles blood was drawn from the ventricle into a syringe moistened with a 1.5 per cent. sodium citrate

solution, 1 cc. of blood was injected into 19 cc. of 1.5 per cent. sodium citrate, the corpuscles then washed four times, centrifuging at low speed to just sediment the corpuscles and the final suspension being brought to 5 per cent. in normal saline. For the serum, blood was run into small test tubes and kept at room temperature over night.

The serum was diluted for all the experiments 1 : 4 with normal saline; the corpuscle suspension being 5 per cent. as stated above. Agglutination tests were made in the hanging drop according to the method outlined by Ascoli (6). No agglutination was observed in any of the serum-corpuscle combinations.

In the tests for the possible hemolytic action of the sera of any one of the four birds, sixteen hemolysis tubes were used in each experiment. Four of the tubes were controls, having the own corpuscles added to the serum, the other twelve were all the possible combinations. One cc. of the 5 per cent. corpuscle suspension was rapidly run into 1.5 cc. of the serum dilution. The tubes were then shaken, placed in the incubator at 38.5° C. for two hours and shaken again every quarter of an hour during this interval. The tubes were then placed in the ice-box at + 9° C. over night and observed the following morning. Furthermore a 5 per cent. corpuscle suspension in saline was always preserved to the end of the period of observation.

The table given demonstrates the results obtained more clearly than any written description. The greater tendency of the Sebright cock and capon serum to hemolyze the Leghorn corpuscles may be noted, but attention must be drawn to the fact, that this is not a constant phenomenon and that in the case of the Sebright capon serum the own corpuscles are hemolyzed to approximately the same degree.

In a single experiment not recorded in the table, the sera of all the birds was diluted 1 : 40 with normal saline. At this degree of dilution distinct hemolysis was observed in the tube having Sebright capon serum and Leghorn capon corpuscles. There was not even a trace of hemolytic action to be noted for any of the other serum-corpuscle combinations.

The different action of the sera tested does not prove an actual difference in the tissues but it is of interest to note the

SERUM.

	Sebright ♂.				Sebright Capon.				Leghorn ♂.				Leghorn Capon.			
Corpusc.	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Sebr. ♂	−	+?	−	−	++	o	++	++	+?	+?	−	+	+?	+?	−	−
Sebr. c	o	o	−	−	+++	o	++	++	+	+?	−	++	+	++	−	−
Legh. ♂	+++	+++	−	−	++	o	+	++	+?	−	−	−	+?	+?	−	−
Legh. c	+?	+?	−	−	+?	o	++	+++	+	+?	−	+	−	+?	−	−

1, 2, 3, 4 are the four separate experiments referred to in the text.
o = no tube. − = no hemolysis. +? = very faint traces of hemolysis. + = hemolysis distinct. ++ = hemolysis definite. +++ = entire tube hemolyzed.

parallel between the hemolytic action of some of the Sebright sera on Leghorn corpuscles and the non-success of Sebright grafts in Leghorns except in a very small percentage of cases.

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CONFIGURATIONS OF BIVALENTS OF HYACINTHUS WITH REGARD TO SEGMENTAL INTERCHANGE.

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INTRODUCTION.

In the majority of the flowering plants examined by the writer, and apparently also in most of those investigated in this respect by others, the homologous chromosomes, which form bivalents at the reduction metaphase, are joined only at the extreme ends. As examples, Canna and Datura may serve, in which this rule holds in the triploids as well as in the diploids. In the largest bivalent of *Uvularia*, however (Belling, 1926), there are additional points of junction (nodes) not at the ends. The short and medium chromosomes of *Uvularia* seem usually to be connected at or near the constriction, and the same is the case with the short and medium chromosomes of *Hyacinthus* (Belling, 1925). These will not be further considered here.

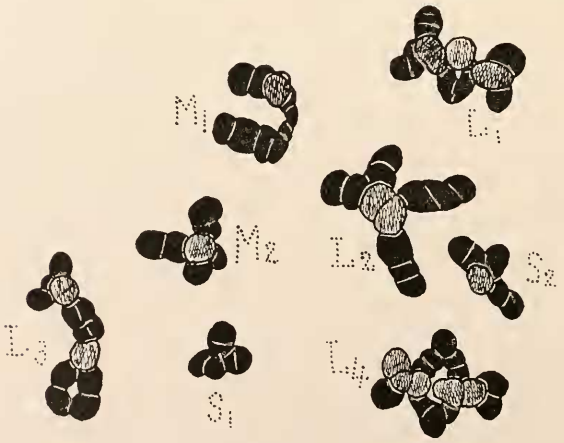

FIG. 1. Camera drawing of the eight bivalents of the diploid hyacinth, squeezed from a pollen-mother-cell. The four large bivalents are alone considered here. They are described in the text.

But the four large bivalents of *Hyacinthus* show, like the large bivalent of *Uvularia*, many connections not at the ends (Fig. 1). It has been pointed out in regard to *Uvularia* (Belling, 1926) that the simplest hypothesis is that these connections (nodes) not at the ends represent places where two of the four chromatids have undergone segmental interchange by fracture and recombination. In *Hyacinthus* it can apparently sometimes be seen with the microscope that two of the four chromatids are bent back at a node, so as to continue along the same sides of the bivalent (Fig. 3). It has also been shown that the homologous chromosomes of the rings and V's formed by the large bivalent of *Uvularia*, acted when separating as if they were not merely twisted across one another, but had undergone a process which produced some interlacing of chromatids at the nodes. This would prevent the simple untwisting of the homologues at the anaphase, and such untwisting has been shown not to occur in *Uvularia* (Belling, 1926). This would also lead (as has been abundantly shown by Janssens, 1924, and others, in animals) to the separation of whole upper and lower halves of vertical rings and V's, and to one chromatid passing up and one down from both sides of horizontal rings or V's, which may get smaller as the process advances, without opening up. This is what takes place in *Uvularia*, and apparently also in *Hyacinthus*.

In *Hyacinthus*, as already stated, the homologues are not always connected at one or both of the extreme ends, but are connected at other places (nodes). This is especially the case with the four long chromosomes. Hence a study of these may show, by the nature of their configurations and their mode of separation at the reduction metaphase, whether the nodes correspond to what would be expected if they were due to segmental interchange between chromosomes (crossing-over of genes).

If the nodes in the long bivalents of the hyacinth are due to segmental interchange, the following phenomena should be observable: (1) the nodes should occur at *different points* in the bivalents in different cases; (2) these nodes should be at equal distances from the ends of both homologues; (3) the nodes should be visible at the late prophase (diakinesis stage or earlier) as well as at the metaphase; (4) the horizontal rings or V's

should split into separate chromatids, while the vertical rings or V's should divide into upper and lower halves, without separating into chromatids; (5) the numbers of bivalents with one or two nodes should permit of a calculation of the numbers of chromatids with segmental interchange at no, one, or two points, which should possibly more or less resemble the occurrence of no, single, or double crossing-over in *Drosophila*. In such a calculation, the numbers of chromatids with no segmental interchange would be equal to twice the cases of single nodes plus the number of cases of double nodes. The total number of chromatids with one point of interchange would be got by adding twice the number of bivalents with single nodes to twice the number with two nodes. While the chromatids with two points of interchange are equal to the number of bivalents with two nodes. It should be possible to test these five points.

(It seems obvious that a junction of homologous chromosomes at the ends has no relation with segmental interchange. It is probably otherwise with junctions at the point of constriction of the chromosome, where segmental interchange may well take place.)

It was for the purpose of testing this hypothesis that the present study was made.

Large Bivalents of *Hyacinthus*.

The variety of *Hyacinthus orientalis* investigated was one of those formerly studied (Belling, 1925), and was chosen because it could be readily identified by the flowers, and had marked characters even in the bulbs. This was the diploid clone called "Yellow Hammer." The bulbs were obtained in October, and put into water during that month and the next. Division of the pollen-mother-cells usually accompanied the development of the first roots. The pollen-mother-cells were instantaneously fixed by being squeezed out from the anthers into iron-acetocarmine. The chromosomes were observed with Zeiss' water-immersion objective 70, yellow-green light, and a water-immersion condenser.

At the first metaphase in the pollen-mother-cells (Fig. 1) the four long chromosome pairs commonly assume one of six different

configurations (Diagram I.). Three of these are shown in Fig. 1, where the cross (L_2) is near the center, two single rings with

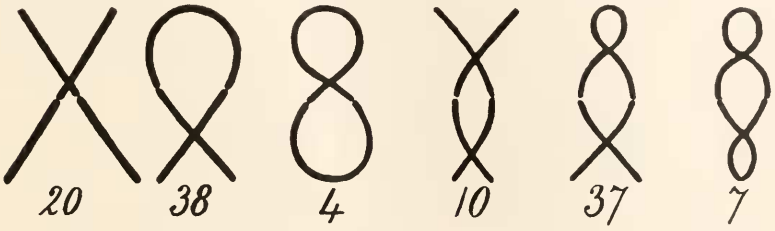

DIAGRAM 1. Numbers of different configurations of large bivalents. The cross, single ring and V, and figure of 8, have one node; while the ring and two V's, the double ring and V, and the triple ring have 2 nodes.

double V's are on the right (L_1 and L_4), and a double ring and V on the left (L_3). Four forms in the late prophase are shown in Fig. 2. They are: (*a*) the ring and V; (*b*) the double ring

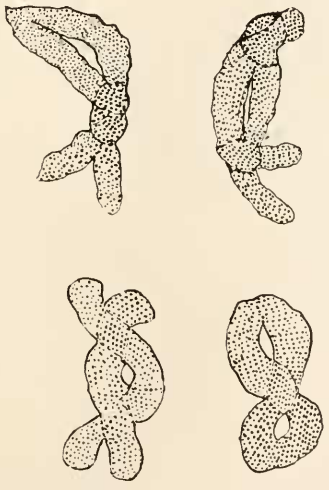

FIG. 2. Four camera drawings of large bivalents at the late prophase. (This stage is difficult to obtain.)

and V; (*c*) the ring and two V's; and (*d*) the figure of 8. The cross and the triple ring were not found free, the bivalents being usually clustered at this stage. Several configurations are drawn in Fig. 3. It may be specially noticed that the four examples of the cross shown in Fig. 3 have the junction in different positions with regard to the chromosome ends. The cross in the middle

has arms which taper to the center, the two homologues having apparently joined at the constrictions. The last two drawings in the lowest line of Fig. 3 are the same bivalent at two different focusses, apparently showing one transverse and one reflexed

FIG. 3. Camera drawings of eight bivalents. The four crosses have the nodes at different points, but always at equal distances from the ends of the homologues. The lowest arm of the second cross in the second line is much foreshortened. The last two figures show the effects of change of focus on the node.

chromatid of one homologue, the same thing being often observable at a different focus in the other homologue also. These are pulled out by the spindle fibers which are attached at the apex of the bend at the median constriction.

One hundred and sixteen of the long bivalents were classified in six groups (Diagram 1), mostly after squeezing chromosomes and cytoplasm from the cell. The results were (Diagram 1): 38 cases of the ring and V; 20 X's; and 4 figures of 8 (totalling 62 with one node); 37 cases of the double ring and V; 10 cases of the ring and two V's; and 7 cases of a triple ring; totalling 54 cases with two nodes. No bivalents with triple nodes were certainly demonstrated. If only two chromatids underwent segmental interchange at any one node, as seems to be indicated

by the microscopical phenomena, we have for the resulting pollen grains and any particular long chromosome, on the hypothesis of segmental interchange: no segmental interchange (124 + 54), 178; single point of interchange (124 + 108), 232; and double interchange, 54; out of a total of 464 chromosomes or pollen grains.

This gives in percentages: chromosomes with no interchange 38 per cent.; chromosomes with single interchange, 50 per cent.; and chromosomes with double interchange, 12 per cent. The ratio of single to double interchange on the hypothesis is thus 4.3 to 1.

This is not far from the numbers of no, single and double points of crossing-over given for the first chromosome of *Drosophila melanogaster* by Morgan (1925). The second and third chromosomes of *Drosophila*, however, seem to differ in this respect.

In Figs. 4 and 5, there are examples of the separation of chromatids and homologues which tend somewhat to prove the hypothesis of segmental interchange. In Fig. 4, L_1 is especially instructive. Here on one side of the constriction (and spindle fiber attachment) there was apparently a horizontal ring, and

FIG. 4. Camera drawing of the bivalents in a cell in which the four large ones had not completely separated, the drawing paper being shifted after each was drawn. Some are foreshortened, especially L_2 and L_4.

on the other side perhaps a vertical ring and a small V; or only a large vertical V. The horizontal ring evidently split into two ring chromatids, while the vertical ring remains. In L_3 the constriction is in the vertical ring. (L_2 and L_4 show nothing more.) In Fig. 5, L_1 shows apparently the separation of the

FIG. 5. Slightly more advanced stage than Fig. 4.

parts of a cross, the two horizontal members of which can be seen splitting into chromatids, one passing to each pole.

DISCUSSION.

The following points seem most worthy of notice.

(1) At some nodes it appears as if both chromatids of each homologue could be seen, one obliquely transverse, and the other reflexed. The spindle fiber is often attached (at or) near the node also at the median constriction.

(2) Some rather scanty data seem to show in *Hyacinthus*, what is more abundantly demonstrated in *Uvularia*, namely, that single chromatids of each homologue pass to the poles from both sides of horizontal rings and V's, and that the two chromatids of each homologue remain connected in the separate halves of vertical rings and V's. So the horizontal rings and

V's diminish in size as their chromatids are pulled out by the spindle fibers towards the poles.

(3) The numbers of cases of possible segmental interchange in the chromatids of the large bivalents, calculated from the numbers of nodes, agrees roughly with the numbers of cases of crossing-over found in the first chromosome of *Drosophila*, which is especially favorable for this study.

Some process of segmental interchange seems demanded by the genetic evidence in *Drosophila*, *Zea*, *Lathyrus*, and the other plants and animals which have shown cases of crossing-over. Hence it is apparently the natural scientific procedure to accept segmental interchange as a working hypothesis to account for the nodes and internodes of the chromosome pairs in the *Orthoptera* and other animals, and also in *Uvularia* and *Hyacinthus*. This is the more imperative in that there seems no other available working hypothesis.

Summary.

(1) The four large bivalents of *Hyacinthus* show in 62 cases one node, and in 54 cases two nodes where the homologues cross.

(2) At these nodes it can apparently be seen with the microscope that one chromatid of each homologue passes obliquely across, while the other seems bent back along the other homologue.

(3) The hypothesis of previous segmental interchange at such a point is assumed until a better hypothesis is found.

(4) The numbers of chromatids showing such points of segmental interchange, according to the hypothesis, calculated from the 116 bivalents examined, were 38 per cent. with no interchange, 50 per cent. with one point of interchange, and 12 per cent. with two points of interchange.

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